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Journal of Chromatography A, 1035 (2004) 271–275
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Isotachophoretic separation of alkylsulfonates and determination of methanesulfonic acid as main component and as trace component in pharmaceutical drug substances

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Received 1 December 2003; received in revised form 20 February 2004; accepted 20 February 2004

Abstract

Alkylsulfonates from methanesulfonic acid to decanesulfonic acid were separated by isotachophoresis with conductivity detection in a common electrolyte system at pH 4.8. The electrolyte system consisted of 10 mM HCl buffered with ε-aminocaproic acid (pH 4.8) and 0.1% methylhydroxyethylcellulose (MHEC) acting as the leading electrolyte. The terminating electrolyte was 20 mM caproic acid also containing 0.05% MHEC. Current settings of 250 μA for the first and 50 μA for the second capillary were applied. On one hand, the method was applied to the determination of the content of methanesulfonate as the salt forming agent (mesilate) in a recently registered drug substance. The results obtained by ITP were compared with an orthogonal titration method. On the other hand, due to the column-coupling configuration of the electrophoretic instrument, the method could be extended to the trace determination in the ppm range in order to monitor methanesulfonic acid as an impurity in a drug substance. The validation confirmed the linearity of the method between 1 and 10 mg/l, limits of detection and quantification below 1 mg/l, recovery rates from 92.4 to 95.4%, and repeatability with a R.S.D. of 3.8% (six runs with a 4 mg/l spiked sample). Finally, three batches of a newly produced drug substance could be checked for methanesulfonic acid giving results of below 0.0014% (concentration related to the drug substance).

Keywords: Isotachophoresis; Pharmaceutical analysis; Alkyl sulfonates; Methanesulfonic acid

1. Introduction

Alkylsulfonic acids and/or alkylsulfonates are widely used in the chemical industry, mainly as catalysts in esterification, condensation, and alkylation processes [1,2].

Due to their role as anionic surfactants, alkylsulfonates can be found in commercial detergents, cosmetics, and hygienic products.

In the pharmaceutical field, methanesulfonic acid and to a lower extent ethanesulfonic acid are used to form salts with a chosen drug substance. Mesilates (salts of methanesulfonic acid) are highly water-soluble and have no tendency to form hydrates. More than 30 registered drugs on mesilates basis are known [3].

Directly linked with mesilates is that salts may become potentially contaminated by mesilate esters, especially when prepared or recrystallized in an alcoholic environment (alcohols easily form esters with the corresponding acid). Methyl methanesulfonate and ethyl methanesulfonate show sufficient evidence in experimental animals for carcinogenity. Both are direct acting alkylating agents which are mutagenic in a wide range of in vivo and in vitro test systems [4]. Teratogenic and reproductive effects were also reported [4].

From the analytical point of view, two different application areas are obvious. First, is the determination of the salt forming agent, which means the content of the alkylsulfonates when used as counter ion to the drug substance. This analytical issue is characterized by a relatively high concentration of the analyte (salt form) usually in the percent range, consequently easy and fast analytical methods, e.g. titration are common [5]. The content of sulfonic acid can be subject of an acid–base titration. Secondly, due to the necessity to monitor sulfonates and/or the sulfonic acid esters in ppm amounts, separation techniques plays a more dominant role. Sulfonic acid esters can be determined by GC [6].

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doi:10.1016/j.chroma.2004.02.061

By nature, sulfonic acids are strong acids in aqueous systems, hence predestined for ion-separation techniques like capillary electrophoresis (CE) and ion chromatography (IC). Surprisingly, only a few publications could be found with respect to the above mentioned two techniques.

IC separations were performed and applied also to some real samples [7–9] including a pharmaceutical compound [10]. However, the separation between methanesulfonic acid and ethanesulfonic acid was not obtained.

Capillary zone electrophoresis in the indirect UV absorption mode represents another alternative. Depending on the electrolyte system and the direction of the electroosmotic flow (EOF) in one run, the separation of C_1–C_7 and C_4–C_{12} alkylsulfonates could be obtained, respectively. Real samples from a petroleum refinery and a cosmetic product were analyzed [11,12].

We favored capillary isotachophoresis (ITP) as another electrophoretic technique for the determination of alkylsulfonates as main and trace constituents mainly for the following reasons:

(i) Conductivity detection as standard detection principle offers a direct approach for the non UV-active alkylsulfonates.
(ii) The EOF plays no role in the separation.
(iii) The enlarged sample load capacity allows work in the low ppm (mg/l) range.

From the apparatus point of view, a column-coupling instrument, introduced by Everaerts et al. [13], provides the possibility of using only the first capillary for the determination of higher concentrated components (salt forming agent) and, if desired, to carry out the determination in the second capillary for the analytes present in lower concentration levels (ppm range) [14].

It is the purpose of this paper to show the applications of capillary ITP during pharmaceutical development in the determination of alkylsulfonates when used as a salt forming agent and when the control of sulfonic acids in the lower ppm range is needed (required) due to the potential forming of their undesired sulfonic acid esters.

2. Experimental

2.1. Instrumentation

An automatic isotachophoretic instrument, the Itachrom II (J&M, Analytische Mess- und Regeltechnik, Aalen, Germany) was used in experiments performed in this work.

The instrument was used in the column-coupling mode: the analysis takes place first in a pre-separation column and then in an analytical column. The first one is provided with a capillary made of FEP, with 800 μm i.d. and a length of 95 mm, the second one is provided with a capillary made of quartz, with 300 μm i.d. and a length of 140 mm.

The instrument is provided with two contactless conductivity detectors, one for each capillary and with a UV detector at the second capillary.

Samples were injected with the aid of a Spark Marathon autosampler. The injection-volume of the instrument is about 30 μl.

For data-evaluation and processing ACES software 1.3 (J&M) was used.

2.2. Chemicals

Chemicals used for the electrolyte system were: hydrochloric acid 30% (Ultrapur), caproic acid (for synthesis) from Merck, aminocaproic acid (for R&D use only) from Fluka and methylhydroxyethylcellulose (MHEC), stock solution 1% from J&M.

Standards solutions used were: methane-, ethanesulfonic acids, and decane-1-sulfonic acid sodium salt (for synthesis) from Merck, butane-, pentane-, hexane, heptane, octane-1-sulfonic acids sodium salt (for ion pair chromatography) from Merck, 1-propanesulfonic acid (for R&D use only) from Fluka, and 1-nonanesulfonic acid sodium salt (for ion pair chromatography) from Fluka.

Water used for all solutions was water for chromatography (specific conductance at 25 °C: ≤1 μS/cm) from Merck.

3. Results and discussion

3.1. Separation of the alkylsulfonates

As the separation in electrophoresis is based on the effective mobility (u_{eff}) of the analytes a closer look at the key parameters like pK_a is obvious.

According to their pK_a-values [15], being in the negative range (around −1) a separation, only based on the optimization of the pH, seems to be unsuccessful. However, the increasing molecular size and the corresponding molecular weight should result in different effective mobilities for each analyte of interest because the fundamental Eq. (1) is valid [16].

$$\mu = 56 + 258|Z|M^{1/2} \quad (1)$$

where μ is the mobility; Z, the charge of the ion; and M is the molecular mass.

We chose for the separation a pH of 4.8 for the leading electrolyte. The electrolyte system was a common one consisting of a chloride based leading electrolyte and with caproic acid as terminating electrolyte [17]. Fig. 1 shows the used electrolyte system and the obtained separation of the C_1–C_{10} alkylsulfonic acids.

3.2. Determination of the salt forming agent

As mentioned in Section 1, sulfonic acids are used as counter-ions of drug substances. When methanesulfonic acid is taken, mesilates are formed.

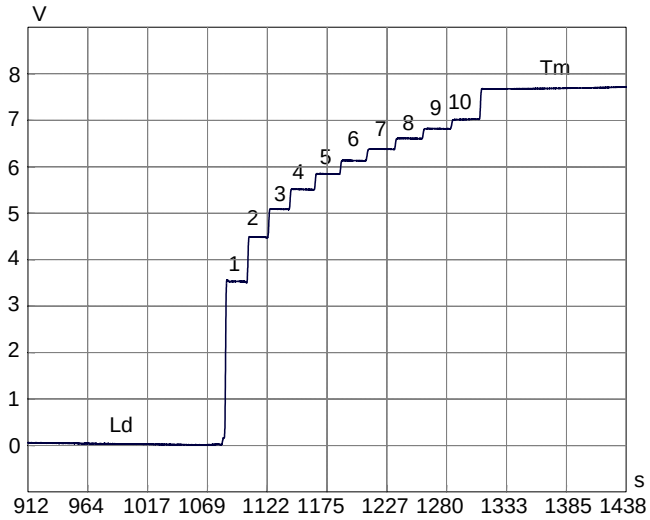

Fig. 1. Isotachopherogram of the separation of a standard mixture of C_1–C_{10} alkylsulfonic acids in the second capillary each with a concentration of 30 mg/l. Leading electrolyte: 10 mM HCl, ε-aminocaproic acid, pH 4.8, 0.1% methylhydroxyethylcellulose (MHEC). Terminating electrolyte: 20 mM caproic acid, 0.05% MHEC. Injection 30 μl, $I_1 = 250$ μA, $I_2 = 50$ μA. Analytes: 1, methanesulfonate; 2, ethanesulfonate; 3, propanesulfonate; 4, butanesulfonate; 5, pentanesulfonate; 6, hexanesulfonate; 7, heptanesulfonate; 8, octanesulfonate; 9, nonanesulfonate; 10, decanesulfonate; Ld, leading; Tm, terminating.

Being a strong acid, the content of the mesilate can be subject of an acid–base titration method [5]. Such a titration is characterized by its shortness and ease of operation. Nevertheless, a titration requires a relative high amount of sample and additionally, reports a sum parameter (e.g. all titratable acids). This impelled us to apply a selective separation method for the determination of the mesilate content in a drug substance. Fig. 2 shows a representative isotachopherogram of such a determination.

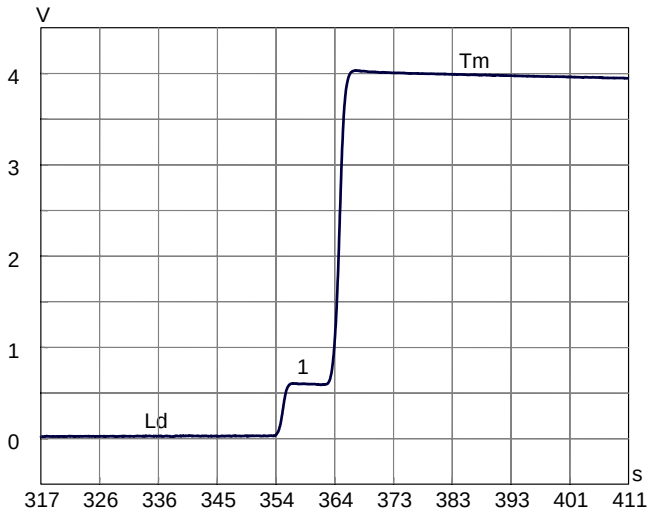

Fig. 2. Determination of the salt forming agent in a drug substance in the first capillary. Electrolyte system of Fig. 1 was used. Injection 30 μl, $I_1 = 250$ μA. Analyte: 1, methanesulfonate (mesilate) at 45.4 mg/l; Ld, leading; Tm, terminating.

Table 1
Comparison of the mesilate determination with ITP and titration taking the mean of $n = 2$ analyses as result

	Batch 13		Batch 14		Batch 16	
	ITP	Titration	ITP	Titration	ITP	Titration
Content of mesilate (%)	16.1	16.3	16.2	16.4	16.0	16.4
Deviation (%)	1.23	–	1.22	–	2.44	–

Due to the fact that the mesilate represents, in this analysis, a main-component, the conductivity detector of the first capillary is sufficient for the detection.

Table 1 illustrates the ITP and titration results of three determinations. The quantification of the analyte was executed by using the average zone length of two measurements of a 60 mg/l standard as reference. These two standards were recorded each time prior to the analysis of the drug substances samples which were also performed in duplicate. The potentiometric titration with sodium hydroxide was performed by using a combined pH-electrode, a 5 ml burette and a suitable titrator. About 200 mg of the test substance were dissolved in a water/ethanol mixture (8:2 (v:v)) and directly titrated. Also, for the titration, the mean value of two measurements was taken as result.

Table 1 clearly indicates a very good correlation of the results between titration and ITP. In this respect, one should also keep in mind the different analytical principles of both techniques.

As advantages, we would like to state the following points highlighting isotachophoresis for the determination of the salt forming agent, when compared to a titration method. ITP is a selective separation method and requires a 10 times lower sample amount.

Mainly, the last point could be of high interest in pharmaceutical development, especially in early phases when the amount of drug substance is limited.

Due to the fact that the ITP analysis is only performed in the first column, the analysis time is about 5 min and thus, in the same range as titration.

Table 2
Parameters of the regression line and obtained LOD and LOQ according to DIN 32645

Linear regression	$y = ax + b$
Concentration range (mg/l)	1–10
Number of points	20
Slope a	1.4461
Standard deviation of a	0.0127
Intercept b	0.4667
Standard deviation of b	0.0788
Significantly different from 0	Yes
Correlation coefficient R	0.9993
LOD (mg/l)	0.25
LOQ(1.5 s) (mg/l)	0.71
LOD relative to the drug substance (%)	0.0005
LOQ relative to the drug substance (%)	0.0014

3.3. Determination of methanesulfonate in the ppm range

Our second application was aimed at the control of methanesulfonate in a synthesis process where the content of sulfonic acid, when used with ethanol, must be controlled due to the potential forming of undesired ethyl methanesulfonate.

A closer look to the reachable limits of detection (LODs) and limits of quantification (LOQs) was, therefore, obviously required.

The common approach using the signal to noise ratio [18] cannot be applied in ITP. We favored the calculation of the LOD and LOQ from the calibration line [19–21].

In our particular case, we followed the DIN 32645 [22]. Consequently, we injected standard solutions from 1 to 10 mg/l of methanesulfonate for recording the calibration line, simultaneously covering in this way the validation parameter linearity. Table 2 gives the obtained regression line confirming the linearity of the method in the mentioned concentration range and the results of the calculation according DIN 32645.

The LOQ resulted from the accurate zone length measurement capability of the conductivity detector. We found that a zone length of 1.5 s is sufficient for a precise zone determination. Therefore, the LOQ of methanesulfonate was adjusted to a zone length of 1.5 s.

Relating the above listed concentrations to the amount of drug substance used in our experiments (50 mg/ml) resulted in a working range from 0.0014 to 0.02% for which methanesulfonate can be checked in the drug substance. Fig. 3 illustrates such a check for methanesulfonate in 50 mg/ml drug substance. For the ppm application another conductivity detector for the second capillary was used.

Obviously, by comparing the detection signals of Figs. 1 and 3 the lower range of the new detector can be noticed.

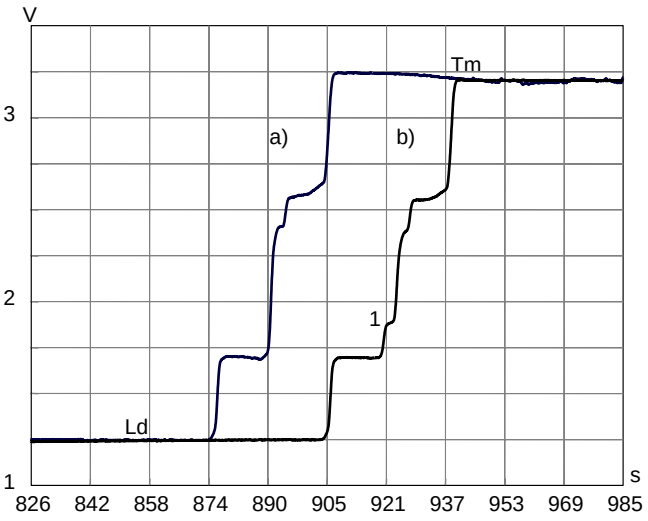

Fig. 3. Determination of methanesulfonate in a drug substance. (a) Drug substance, (b) drug substance spiked with 0.004% methanesulfonate. Electrolyte system of Fig. 1 was used. Injection 30 μl, $I_1 = 250$ μA, $I_2 = 50$ μA. 1, Methanesulfonate; Ld, leading; Tm, terminating.

Table 3
Repeatability of the 0.008% spiked sample

Measurement	Zone length (s)	RSH
1	5.80	0.299
2	6.36	0.283
3	5.96	0.315
4	5.92	0.306
5	5.80	0.311
6	5.76	0.296
Mean (s)	5.93	0.302
S.D. (s)	0.22	0.012
R.S.D. (%)	3.76	3.84

For quantification purposes the linearity (working range of the detector) should be checked prior to the analysis.

3.3.1. Validation

In addition to the already performed validation parameters like linearity, LOD, and LOQ, we evaluated the method with respect to repeatability (precision), recovery (accuracy), and stability of the solutions.

The repeatability was tested by executing six injections of the 0.008% spiked sample in intermediate succession by the same operator. The results of the quantification (zone length) and the identification (RSH) are summarized in Table 3.

In order to evaluate the recovery, three concentration levels were spiked to the drug substance and analyzed three times each. The quantification was done with the already recorded calibration line (Table 2) after the average of the three runs was calculated. For the concentration levels 0.004, 0.008, and 0.012% recovery rates of 92.37, 95.39, 93.60% for methanesulfonic acid were found, respectively.

We paid also attention to the stability of the solutions. For this reason, the sample with the concentration level of 0.012% was again injected after two days of storage in brown glass vials at room temperature. The obtained average zone length (mean of three runs) differed only by 0.4% compared to the time of preparation.

Finally, we checked three newly produced batches of drug substance for the content of methanesulfonic acid. In all batches we could not find the analyte with our method which meant that the content of methanesulfonic acid was below 0.0014% (14 ppm) in each sample.

4. Conclusion

It has been shown that ITP is a powerful tool for the analysis of alkylsulfonates in a broad concentration range, e.g. from the percent down to the ppm range. We chose the alkylsulfonates as an example of polar and non UV-active analytes. It can be assumed that the described methods can be transferred to other representatives of this class, e.g. organic acids and inorganic anions which also need to be monitored

in pharmaceutical drug substances. Due to the fact that no sample preparation is needed and the analytical results are obtained within 20 min, ITP is also suitable for in-place quality and process control.

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